5-2012

Molecular Modeling of Estrogen Receptors

Chelsea Knotts
cknotts@utk.edu

Molecular Modeling of Estrogen Receptors

Chelsea M. Knotts

Introduction

Estrogen receptors are members of the nuclear receptor superfamily, enzymes involved in signal transduction. [1] They are transcriptionally activated by the binding of ligands to their ligand-binding domain. When nuclear receptors become transcriptionally active, they control the activity of specific genes networks during development, differentiation, and homeostasis.[1]

17 ß-estradiol, or estrogen is the endogenous ligand that binds to an estrogen receptor, transcriptionally activating it. [2] When estrogen activates an estrogen receptor, it plays an important role in bone maintenance. In the cardiovascular system, it has been shown to have cardio protective effects. Estrogen is also an important ligand in the brain, affecting the activity and connectivity of neuronal populations. The modulation of the brain in this way is important for regulating reproduction, mood, behavior, and gonadotropin production and release. Recent studies have shown that estrogen may also affect brain function in events including learning and memory. [3]

Estrogen receptors are composed of eleven major alpha helices. These helices are arranged in three layers, antiparallel to each other. [4] These receptors can be divided into 6 functional domains, denoted A-F. [3, 5] Region C is the most conserved region and contains the DNA binding domain. This domain has nine cysteine residues that are always conserved in nuclear receptors. Eight of the cysteine residues organize around two type II zinc clusters, or zinc fingers. The zinc fingers are involved in specific DNA binding and receptor dimerization. [3, 6]

The E region of nuclear receptors contains the ligand-binding domain, the second highly conserved domain in this receptor family. [5, 7] This domain is multifunctional and facilitates the receptor's ability to activate transcription and cross

talk with other signal transduction pathways. [7] The ligand-binding domain is part of a hydrophobic cavity, buried in the core of the estrogen receptor. [4]

The ligand-binding domain is necessary for the binding of the ligand to the estrogen receptor, the localization of the receptor in the nucleus and dimerization of the receptor. In addition, the binding domain contains a transactivation function, AF-2. This region is composed of an amphipathic alpha helix and is dependent on ligand binding for interaction between the ligand binding domain and co-activators. [7] A second transactivation contains, AF-1, has also been discovered in the A domain of estrogen receptors, a less conserved domain at the N-terminus of the protein. It is possible that AF-1 functions independently of ligand binding, and may be necessary for full activity of the estrogen receptor. [5]

Figure 1: Functional domains of estrogen receptors [8]

Binding of estrogen to estrogen receptors incudes a conformational rearrangement in the ligand-binding domain. This activated structure is referred to as RXR gamma, the active holo-LBD structure, whereas the inactive apo-LBD structure is referred to as RXR alpha. [4] Induced structural changes lead to RXR gamma being a more compact structure than RXR alpha. This is due to H12, the alpha helix that includes the AF-2 transactivation function, folding back towards the ligand-binding domain. This change is the most significant. Additional changes include the flipping of a Ω loop underneath H6, the bending of H3 towards the center of the ligand-binding

domain, the tilting of H11 away from the hydrophobic core of the ligand-binding domain, and the disruption of a salt bridge between H12 and H4. [7]

The conformational rearrangements caused by ligand binding provide a mechanism for transcriptional activation of the estrogen receptor. A ligand is attracted to the estrogen receptor by the electrostatic forces present in the ligand-binding cavity of the RXR alpha form. The movement of the ligand into the binding cavity pushes alpha helix H11 away from the cavity, repositioning it to form a bent helix with H10. This conformational change causes H12, the alpha helix containing transcription factor AF-2, to move under H4, causing the interaction between H12 and the Ω-loop to dissolve. The Ω-loop then flips over underneath H6, moving H3 with it. Finally, H12 moves over the ligand-binding domain, sealing the cavity. [7]

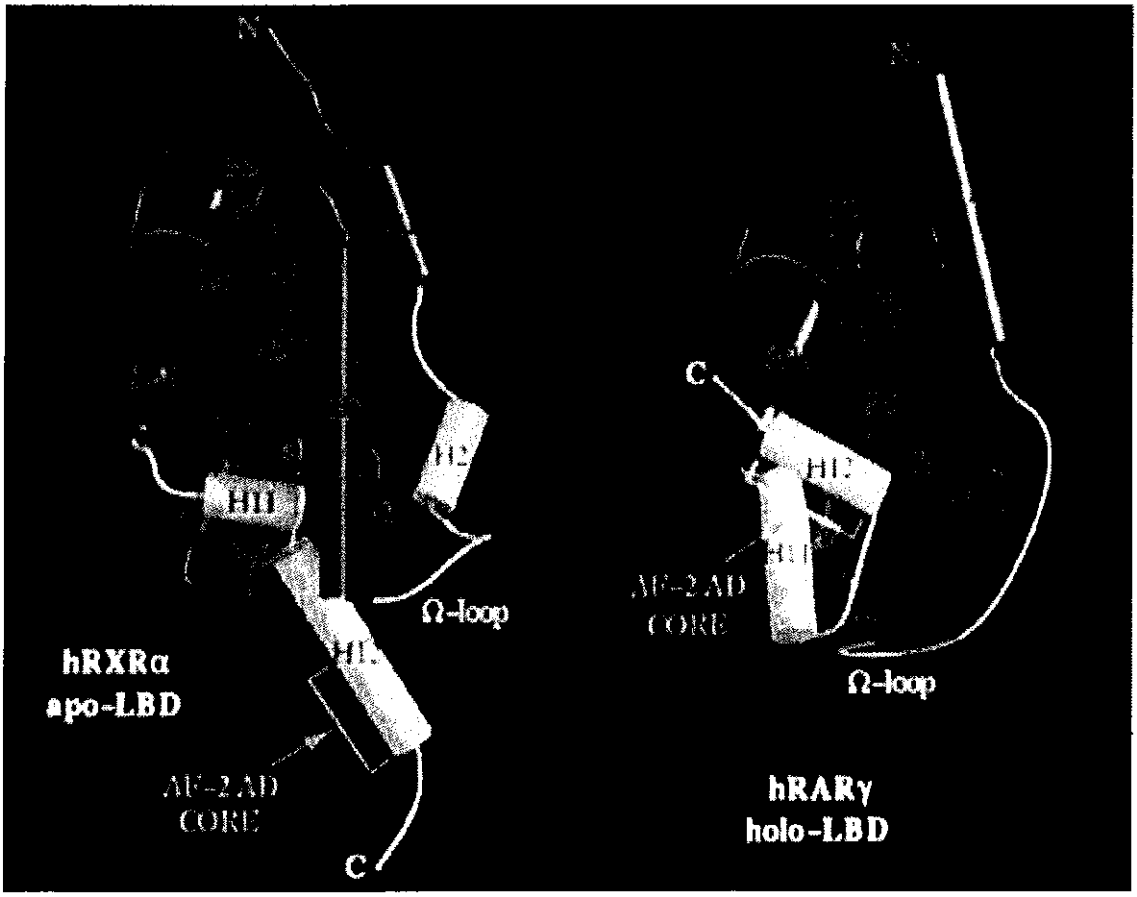
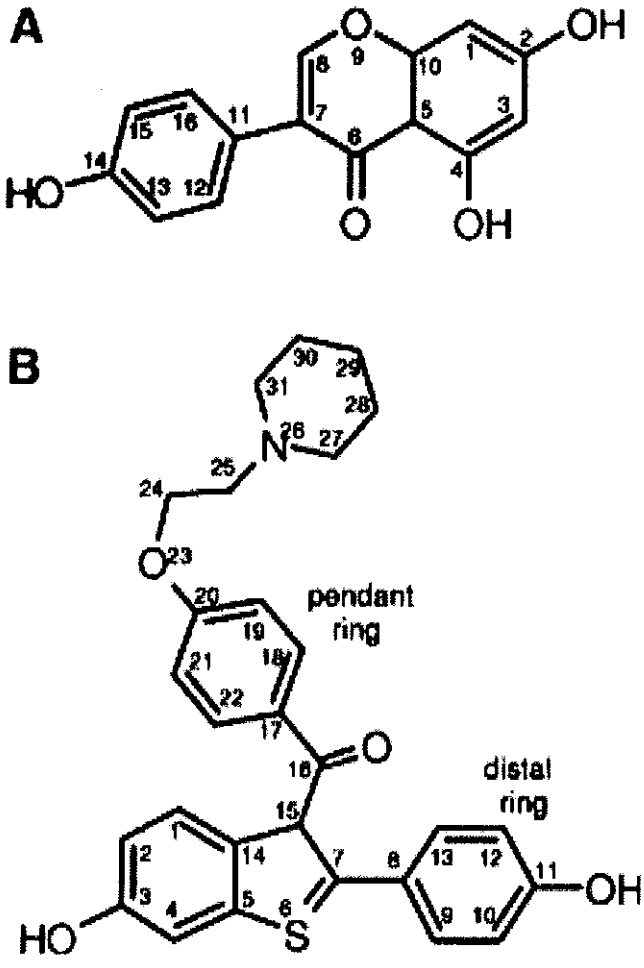


Figure 2: Apo-ligand-binding Domain (Without ligand) and holo-ligand-binding domain (With ligand) [7]

Because of the large size and shape of the estrogen receptor ligand-binding cavity, Estrogen receptors are able to bind a range of ligands with significantly differing structures. This, in addition to the variety of known ligand-binding modes suggests that ligand selectivity is generated through many different interactions with ligand-binding domain residues. [4]

The positioning of the H12 helix determines whether the estrogen receptor is in an agonist or antagonist conformation. Each ligand that binds to an estrogen receptor interacts uniquely with the residues in the ligand-binding domain cavity, producing the movement of H12. Genistein, an agonist of estrogen receptors, is completely buried within the hydrophobic cavity in a manner comparable to the binding of 17- β estradiol. This allows the H12 helix to seal the ligand-binding domain cavity and transcription to occur. Raloxifene, an antagonist of estrogen receptors, protrudes from the ligand-binding domain cavity, preventing H12 from sealing the cavity, and transcription from occurring. [4] In addition to agonists and antagonists, there is also a third category of estrogen receptor ligands called selective ER modulators (SERMS). These ligands can act as agonists or antagonists, depending on the type of cell, the type of promoter, and the ER isoform targeted. [4]



ure 3: Structure of A: Genistein and B: Raloxifene[4]

Figure 4: Ligand-binding domain bound to Genistein (top) & Raloxife (bottom) [4]

In 1996, a new class of estrogen receptors was discovered. This class was named estrogen receptor beta, and the original class of estrogen receptors was named estrogen receptor alpha. [8] While ERα is located on chromosome 6, ERβ is located on chromosome 14. [9] Though ERα is composed of 595 amino acids, ERβ is composed of only 530. [8]These two classes share only 47% overall sequence identity with little homology between the N-terminal transactivation function located in domain A. [4] In contrast, the DNA binding domain is highly conserved, only differing by 3 amino acids, and the ligand-binding domain shows 59% homology. [3, 9] When complexed with a

ligand, ERα and ERβ have the ability to form a homo-dimer or a hetero-dimer, complexing with each other. [3]

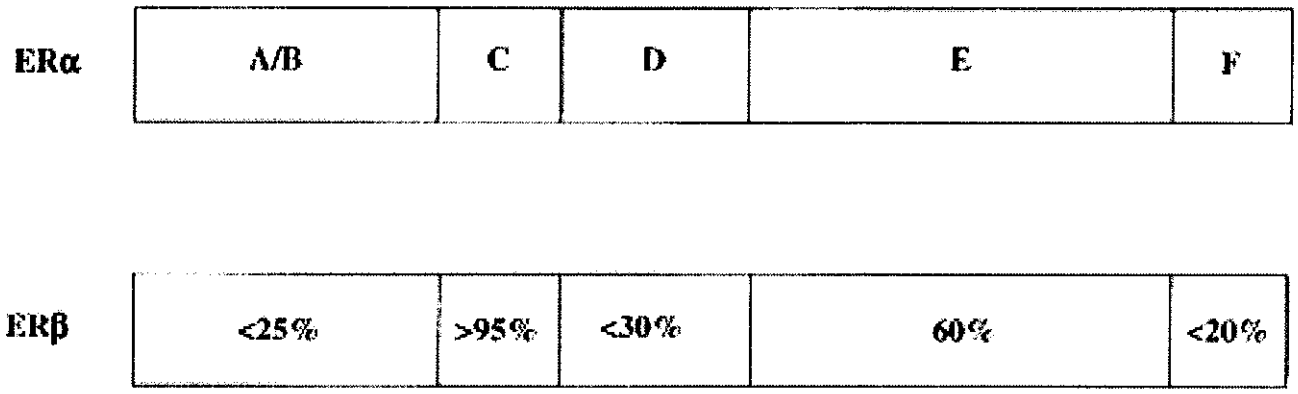


Figure 5: Functional domains of estrogen receptors and homology between ER-Alpha & ER-Beta [3]

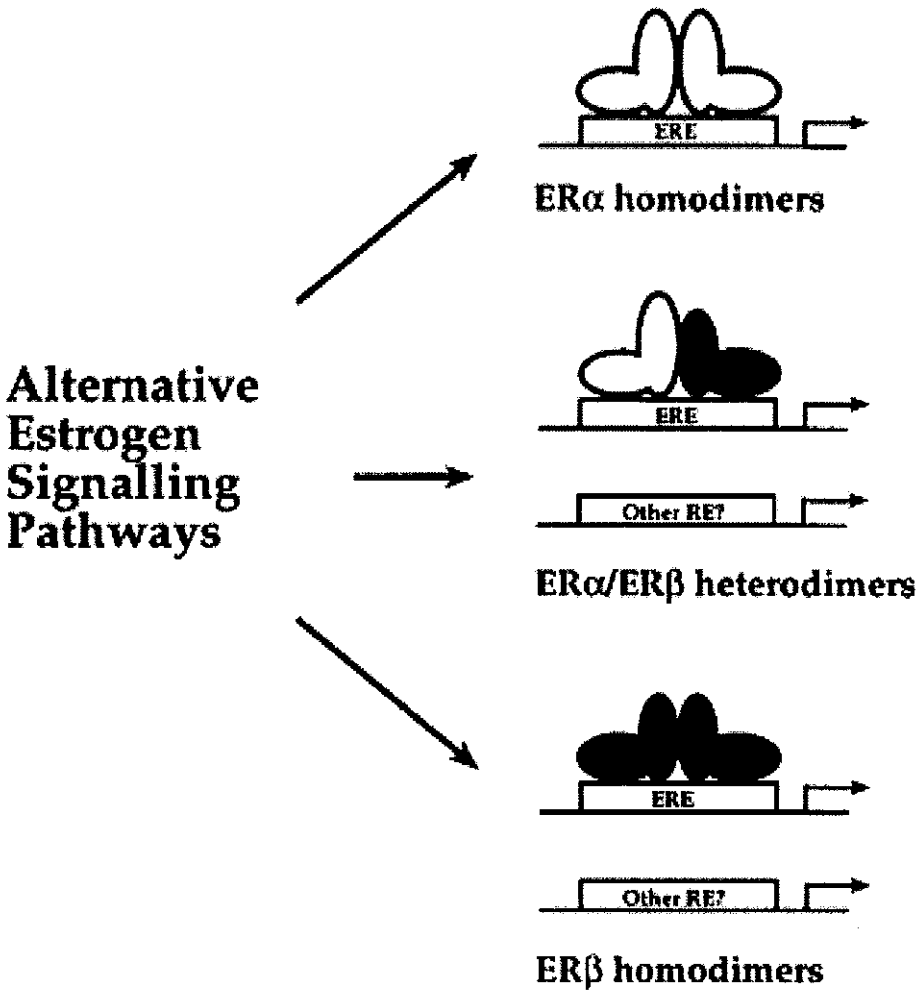


Figure 6: Estrogen Receptor Dimers [3]

The only significant difference in the three-dimensional structure of ERα and ERβ is the position of alpha helix H5. This change effects the position of Leu324 (372 in alpha) and Val328 (376 in alpha), causing the hydrophobic groove to be slightly wider than the groove in ERα. The invariant structure of ERα and ERβ explain why the majority of estrogen receptor ligands bind to both isoforms with similar affinities. However, the changes in position of these two amino acids may explain higher binding affinities for some ligands, such as Genistein, in ERβ [4]

Though the three-dimensional structure of the estrogen receptor is highly conserved between ERα and ERβ, there are two high sequence variability regions. The first is at the N-terminus before alpha helix H3, and the second is a region of 35 residues near alpha helix H10. This second region is at the top of the ligand-binding domain, but only two changes, at position 336 (384 in ERα) and 373 (421 in ERα) are within the ligand-binding cavity. [4]

The Leucine residue at position 384 in ERα is switched to a methionine residue at position 336 in ERβ. This residue sits above the ligand and forms the pocket on the β-face of the ligand-binding cavity. When complexed with an agonist such as Genistein, interacts with this methionine residue through van der Waals interaction. This interaction occurs with the flavone ring on the Genistein ligand. In addition, though methionine is a relatively hydrophobic amino acid, in the protein structure, the sulfur atom has a slightly polar character that is involved with hydrogen bonding interactions that may stabilize ligands. [4]

The second substitution within the ligand-binding domain is a methionine at position 421 in ERα for an isoleucine at position 373 in ERβ. This residue substitution is at the start of alpha helix H8. This residue is part of the α-face of the cavity below the D-ring and is between His475 (524 ERα) and Phe377 (425 in ERα). The migration

of the methionine, a slightly polar R-group, from the α-face of the ligand-binding cavity to the β-face may enable ERβ to accommodate more polar ligand substituents at that end of the cavity. [4]

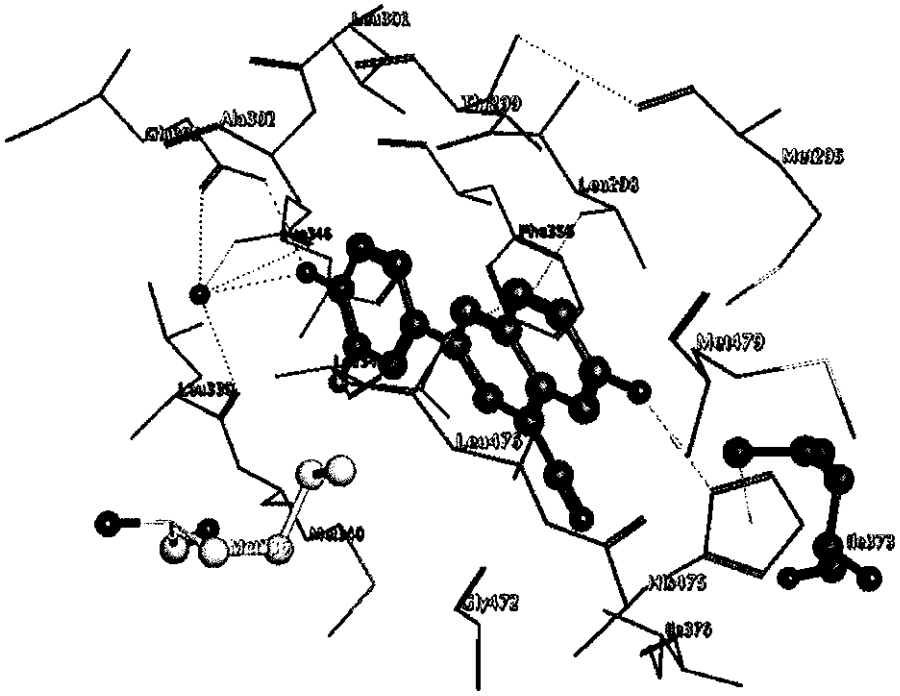


Figure 7: Beta Ligand pocket with Met 336 in turquoise and Ile 373 in fuchsia

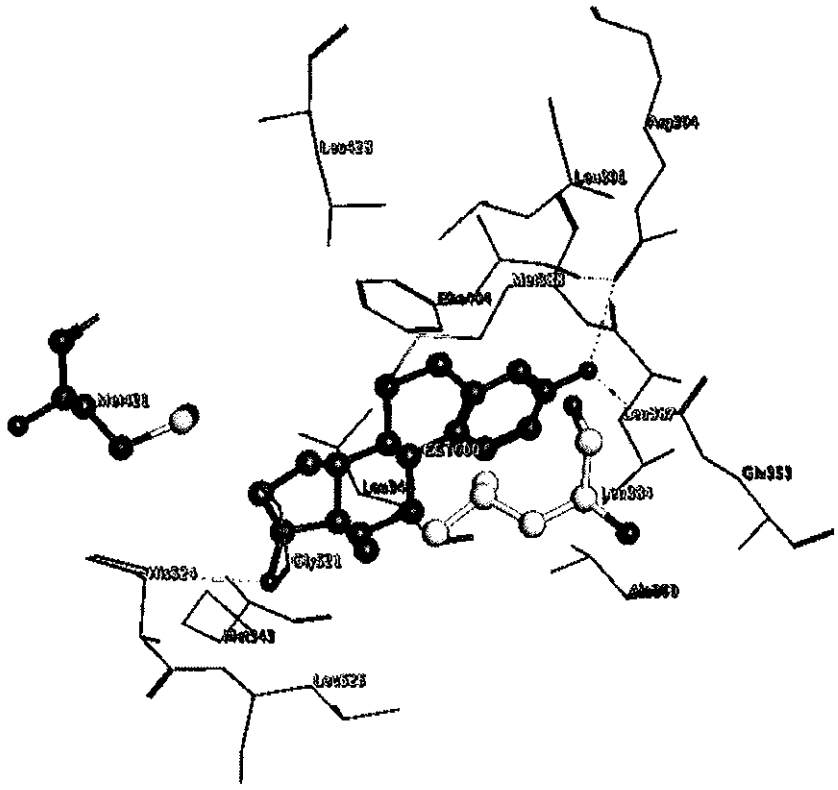


Figure 8: Alpha Ligand pocket with Leu 384 in turquoise and Met 421 in fuchsia

Functionally, ERα and ERβ bind ligands with similar affinities. One difference is that ERβ forms antagonist conformation more easily than ERα. This is due to the instability of the alpha helix H12 in ERβ as compared to ERα when in the agonist conformation. A lysine residue at 300 in ERβ replaces an asparagine residue at 348 in ERα. This change prevents a hydrogen bonding interaction with a tyrosine residue at position 398 (537 in ERα), destabilizing the alpha helix. [4] This destability may lead to some ligand binding differences. For example, in one study done on ERα and ERβ ligand binding differences, Bisphenol AF, a full agonist for ERα, functioned as a highly specific antagonist for ERβ. [1] In addition to this functional difference, depending on the tissue and transcription target, ERα and ERβ were shown to have different transcriptional affects. For example, in breast tissue, ERα and ERβ, when complexed with estrogen, were shown to signal in opposite ways from an AP1 site. Estrogen activated transcription in the presence of ERα but inhibited transcription in the presence of ERβ. [10]

In addition to structural and functional differences between ERα and ERβ, there are also differences in tissue distribution. ERβ has a wider but overlapping tissue distribution than ERα, including the gastrointestinal tract, lung, and brain. ERβ has also been identified in multiple types of tumors that were thought to be Estrogen receptor negative, due to assays that only tested with ERα antibodies. These tumors include colon, esophagus, stomach, brain, lung, prostate, testis, pancreas, and blood vessels. Lastly, while ERα is traditionally localized to the nuclei of cells, ERβ has been detected in both the nucleus and the cytoplasm of normal and cancerous cells. [4, 8]

This research project involves environmental estrogens and their binding to estrogen receptor alpha and beta. Environmental estrogens are a large and structurally diverse group of compounds that can mimic and in some cases antagonize

the effects of endogenous estrogens. Often, they are referred to as endocrine disruptors. [11]These compounds are split into two categories, xenoestrogens and phytoestrogens. Xenoestrogens are a group of synthetically made compounds that include pesticides and industrial pollutants. They often have a negative effect on human and wildlife populations. Phytoestrogens, in contrast, are naturally occurring compounds with estrogenic properties. They are often found in plants or in the metabolites of bacteria and fungi. These compounds may have positive effects, protecting against some forms of cancer, cardiovascular disease, and osteoporosis. [11] Because the effects of endocrine disruptors can cause many developmental, neuronal, and immune diseases, identifying and regulating these substances is important to human and animal welfare. In addition, because of the differences between ERα and ERβ, it should be possible to develop ligands specific to the estrogen class. [9]

Until now, endocrine disruptor identification was difficult because numerous chemicals need to be tested for their potential as disruptors, a slow and expensive process, and many discovery methods do not identify chemicals as endocrine disruptors when they act disruptively in a secondary form. This project addresses these concerns with an integrated and multi-stepped approach. The chemical binding activity of different chemicals with estrogen receptors will be evaluated using a Molecular Operating Environment, or MOE. MOE is a molecular modeling program designed to handle large biological molecules. This research focuses using MOE to obtain results similar to those obtained through experimental testing and to record difference in binding between ERα and ERβ.

Methods

Part 1

A molecular database was constructed in MOE using ligands that had been experimentally tested in ER β and their IC_{50} values recorded. [12] To build this database, each molecule was built in MOE using the Molecule Builder toolbox. After each molecule was built, the molecule was minimized. If any atom had an unnatural charge, the charge was removed. Then, each molecule was saved into the molecular database. The database consisted of 106 ligand structures. Each ligand was built from 1 of 5 different scaffolds: Phenyl Benzisoxazoles, Naphthyl Benzisoxazoles, Naphthyl Benzoxazoles, 5- and 6-Hyxdroxy-2-Phenyl Benzoxazoles, and 7-Substituted 2-Phenyl Benzoxazoles (See addendum at end of thesis) The IC_{50} value for each ligand in ER β was also included in this database, and the molecules were ranked based on their IC_{50} values.

After the database was constructed, a crystallized Estrogen Receptor β structure was downloaded from the protein data bank. The ER β selected was 1YYE, a human estrogen receptor beta complexed with Way-202196, a simplified version of Genistein. [13] The crystallized ligand in this ER β was added as 1 of the 106 compounds in the database. This structure was not used in any correlations but was used to ensure that conditions in ER β were being correctly emulated and ligands were binding as they would *in vivo.* This PDB file was opened in MOE. Because estrogen receptors are dimerized when active, the crystallized structure contained two estrogen receptors and two ligands. One of the dimers was deleted from the structure of the protein, along with it's associated ligand and water molecules.

The protein was then prepared. First, all hydrogen atoms and partial charges were added to the protein structure using the Protonate3D application. Second, the

binding site was identified using the site finder application. MOE found multiple possible binding sites for this protein, but the binding site that contained bound Genistein was the binding site used. After the binding site was located, dummy atoms were placed in the binding site, and the crystallized ligand was deleted.

The molecule was then ready to be docked. To dock the molecular database into the ER β, the selected site of docking was the location of the dummy atoms, and the selected ligands were the molecular database that was constructed. The placement of the ligands was determined using Triangle Matcher and this was rescored using London DG. The placement of the ligands was refined using Forcefield, and again rescored using London DG. The Forcefield placement option was configured so that the protein was tethered and able to move freely, in contrast to the rigid crystallized structure. The tether was set at 20. Lastly, the number of retained placement possibilities per ligand was set at 10 and the simulation was started. During this simulation, MOE tested each ligand in hundreds of different conformations, determined the strength of the interactions between the protein and the ligand in each conformation, and chose the best conformations for each ligand in ER β. This process was repeated multiple times, and each time, different variables in the docking were changed in an attempt to better simulate the *in vivo* conditions of ER β. First, the protein was docked at different tethering strengths: .1, 1, 5, 10, 20, and rigid. Then, the protein was edited to include a water molecule in the ligand-binding pocket. This water molecule is an important molecule for ligand binding in ERα and was thought to also be important to the interactions between the protein and the ligand in ERβ. The protein was again docked using multiple tethering strengths. Next, a histidine residue was protonated, His475. This protonation was also thought to be important to the interactions between the ligand and the protein. The protein was

docked at the same tethering strengths. Lastly, the protein structure was minimized using a MOE application and again, docked at the same tethering strengths.

After each ligand was docked and the score of each ligand conformation obtained, the best conformation of each ligand was isolated from the list of conformations. This conformation was chosen based on the S score: The more negative the S score, the stronger the interactions between the ligand and the protein. Then, each molecule was ranked based on their S score. This database was then merged with the original database created so that the S values could be compared to the IC_{50} values for each ligand. The larger the IC_{50} value is, the weaker the interactions between the protein and the ligand. A correlation plot was drawn comparing the ranking of each ligand based on their IC_{50} value in ER β to their ranking based on their S score in ER β.

Part 2

A second database was constructed in MOE based on an experiment in which compounds were tested in both ERα and ERβ and the K_i values for each was obtained. [14] Because these ligands were more common than the ones in the first database, they were downloaded from the protein database instead of being drawn in MOE. The compounds contained in this database were 17- β estradiol, Diethylstilbestrol, Estrone, Estriol Raloxifene, Tamoxifen, 4-OH Tamoxifen, RU486, and Progesterone. (See addendum at end of thesis)

A second crystallized structure was also downloaded from the protein database, 1ERE. This crystallized structure was a human estrogen receptor alpha, complexed with 17-β estradiol. [15] This protein was prepared in MOE as 1YYE was prepared in part one.

Next, ligands in the second database were docked using MOE in 1ERE, estrogen receptor α and in 1YYE, estrogen receptor β. The docking specifications were the same as in Part 1 of this experiment, but each protein was only tethered at 1, instead of multiple tethering strengths.

After each protein was docked, the best conformation for each ligand in ERα and ERβ was determined based on the S score. The more negative the S score, the stronger the interactions between ERβ and the docked ligand. The S scores of each ligand in ERα and ERβ were then compared to the K_i values of ERα and ERβ, respectively, in a correlation plot. The larger the K_i value is, the weaker the binding interactions between the ligand and the protein are. Because no activity was recorded for the experimental value of RU486 and Progesterone, these compounds were left out of the correlation plot to prevent skewing of the results.

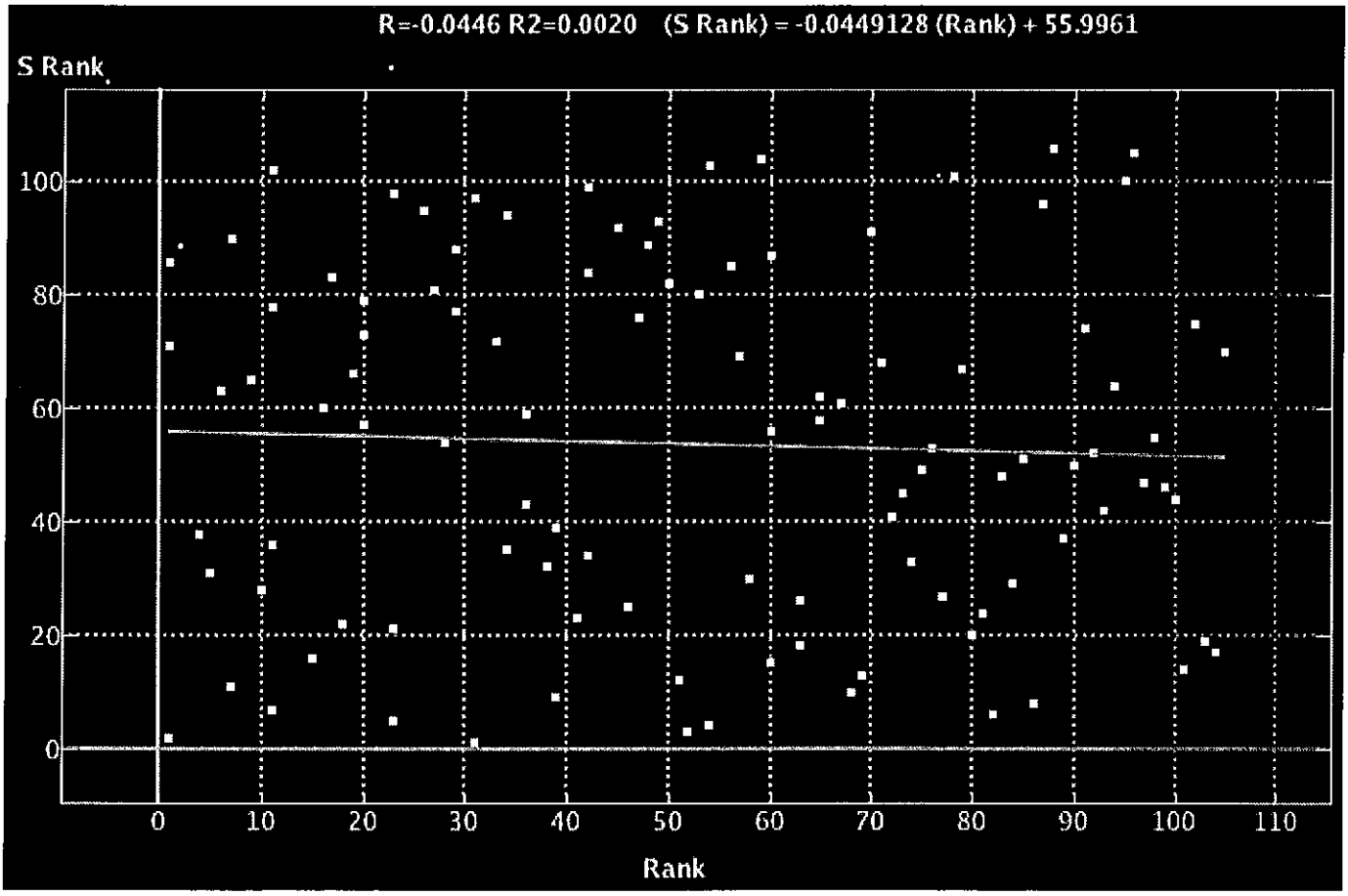
Results and Discussion

Part 1

The best S scores of each ligand and their IC_{50} values are in the below chart. The correlation chart is also below. There was no correlation between the S score and in all docking trials done. Though the regression line showed a slightly negative value with an R-value of -.0446, the points plotted showed no clear patter. These results were unexpected and could be due to the similarity of the structures in the first database. Because the structures are all similar with little structural diversity, the S values for each structure were very similar, and therefore, more difficult to rank. These results may also be due to a problem in the created database.

A last possible cause to explain the results found is that the S score in MOE is not used to differentiate between agonist and antagonist ligands. However, because

an IC_{50} value is a measure of the ligand's ability to inhibit protein function, the experiment determining IC_{50} values was measuring each ligand's ability to antagonize ERβ. Compounds that were considered inactive because of their high IC_{50} values may have skewed the correlation plot; although these ligands were unable to antagonize the protein, they may have bound tightly to ERβ as an agonist, resulting in a higher ranked S-score than IC_{50} value. In future dockings, to remedy this problem, the compounds that were considered inactive could be removed from data set to see if a better correlation can be computed. In addition, future databases drawn could include only known agonists or only known antagonists; a different experimental value could also be used to measure the binding affinities of each compound so that agonists and antagonists would not have to be differentiated.



ure 9: Correlation plot IC_{50} rank vs. S ran

Moe #	IC50	Ranking	ERB Score Tether 20	Ranking
1	3	11	-10.8796	96
2	50	57	-10.3164	102
3	181	83	-12.2958	51
4	105	74	-13.1532	26
5	39	50	-10.9498	93
6	703	95	-11.5644	69
7	157	81	-12.1552	58
8	1600	103	-11.6312	68
9	3660	104	-13.7038	11
10	49	56	-10.955	91
11	6	23	-10.7819	97
12	66	65	-11.1034	85
13	239	87	-12.2324	57
14	59	60	-11.2009	77
15	25	42	-11.066	88
16	16	36	-11.9077	63
17	64	63	-12.2482	56
18	42	52	-12.6116	39
19	963	98	-10.6509	99
20	1.4	1	-13.4715	14
22	87	69	-13.7074	10
23	112	75	-14.9906	1
24	5	20	-13.4265	16
25	3	11	-14.6506	2
26	1530	102	-13.4198	17
27	1050	100	-12.3169	50
28	134	76	-11.1596	80
29	46	54	-13.3503	20
30	15	34	-14.2877	3
31	6	23	-12.9484	31
31	20	39	-13.9249	6
32	718	96	-9.3807	105
33	31	47	-10.6188	100
34	37	49	-10.9127	94
35	20	39	-12.3402	49
36	25	42	-12.389	45
37	33	48	-11.0354	90
38	96	71	-12.2716	54
39	383	92	-11.286	74
40	1.8	5	-13.1562	25
41	2	7	-11.7674	65
42	5	20	-11.6946	66
43	254	88	-10.1264	103
44	3.6	18	-13.2854	23
45	54	59	-10.7286	98
46	138	78	-9.8393	104
47	46	54	-12.382	46
48	10	27	-10.4662	101
49	12	29	-11.0779	86
50	12	29	-11.3509	73
51	8	26	-10.9532	92
52	59	60	-11.1185	84
53	40	51	-11.155	81
54	1040	99	-11.134	83
55	1340	101	-10.8989	95
56	59	60	-11.172	79
57	356	90	-11.9535	62
58	190	84	-11.8308	64
59	95	70	-11.3939	71
60	5000	105	-11.06	89
61	13	31	-12.2608	55
62	11	28	-12.7513	36
63	2	7	-11.1389	82
64	82	68	-11.1974	78
65	79	67	-13.3112	21
66	15	34	-12.277	53
67	13	31	-11.2319	76
68	14	33	-12.9952	30
69	3	11	-13.0378	28
70	3.5	17	-12.2803	52
71	5	20	-13.0191	29
72	3.2	16	-13.3934	18
73	142	79	-12.8196	34
74	3	11	-12.4475	42
75	45	53	-11.3553	72
76	16	36	-11.6621	67
77	23	41	-13.0628	27
78	1.9	6	-12.486	40
79	3.7	19	-12.0075	61
80	2.2	9	-12.4573	41
81	66	65	-12.7461	37
82	201	85	-13.2318	24
83	27	46	-12.091	59
84	235	86	-12.4372	43
85	166	82	-13.9732	5
86	135	77	-13.6246	12
87	313	89	-13.353	19
88	97	72	-12.0861	60
89	366	91	-13.7159	9
90	102	73	-13.435	15
91	1.4	1	-12.7175	38
92	1.4	1	-11.2354	75
93	6	23	-12.3697	47
94	26	45	-12.43	44
95	2.4	10	-11.441	70
96	17	38	-12.9168	32
97	1.5	4	-12.3585	48
98	879	97	-11.0746	87
99	521	93	-14.0868	4
100	533	94	-13.2873	22
101	25	42	-12.8388	33
102	155	80	-13.8392	8
103	52	58	-12.7819	35
104	64	63	-13.8958	7
106	3.1	15	-13.5996	13

Part 2

The correlation plots for the K_i values vs. the S scores of ERα and ERβ are plotted below respectively. In the first correlation plot for ERα, there is a negative correlation between the K_i value and the S score for the ligands. This is opposite of what is expected. However, when Tamoxifen, one of the ligands docked is removed from the correlation plot, the results display a positive correlation as was predicted. One reason for this is that Tamoxifen interaction with estrogen receptors is mediated by cytochrome P450. [16] This was not accounted for in the MOE docking. 4-OH Tamoxifen, a variation of the Tamoxifen ligand that was included in the ligand database, is an active metabolite of Tamoxifen and P450. Though Tamoxifen has a weak affinity for ERβ, the affinity 4-OH Tamoxifen to ERβ is up to 100 times stronger. [17]Because of this, 4-OH Tamoxifen does not skew the results as Tamoxifen does. The R-value for the correlation plot that does not include Tamoxifen was .4806.

In the correlation plots for ERβ, similar results are obtained. When Tamoxifen is included in the results, a negative correlation between K_i values and S score is found. However, when Tamoxifen is removed from the correlation plot, a positive correlation is found. The R-value for the correlation that does not include Tamoxifen was .3482.

The positive correlation in the second part of this experiment verifies that the poor correlation between the ligand S scores and the IC_{50} values in the first experiment was not caused primarily by the conditions of the docking simulation. Using the same ERβ that was used in the first experiment, the second experiment showed that we were able to mimic the conditions of ERβ *in vivo* with enough precision to have a positive correlation between the S scores we obtained and the K_i values that were experimentally determined. Though our modeling of ERβ may have

partly caused the poor correlation between S score and IC_{50} values, other problems must have compounded this issue.

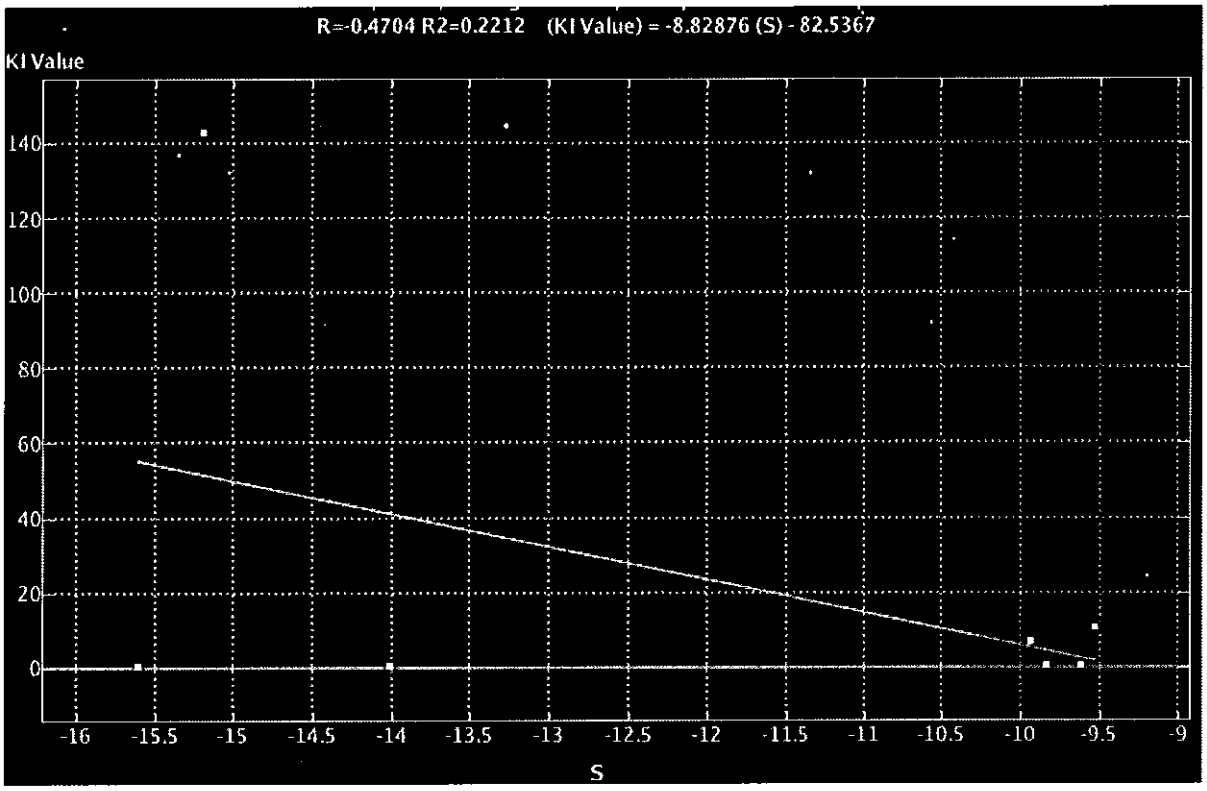


Figure 10: Correlation plot: K_i vs. S for ligands in ER Alpha (Tamoxifen included)

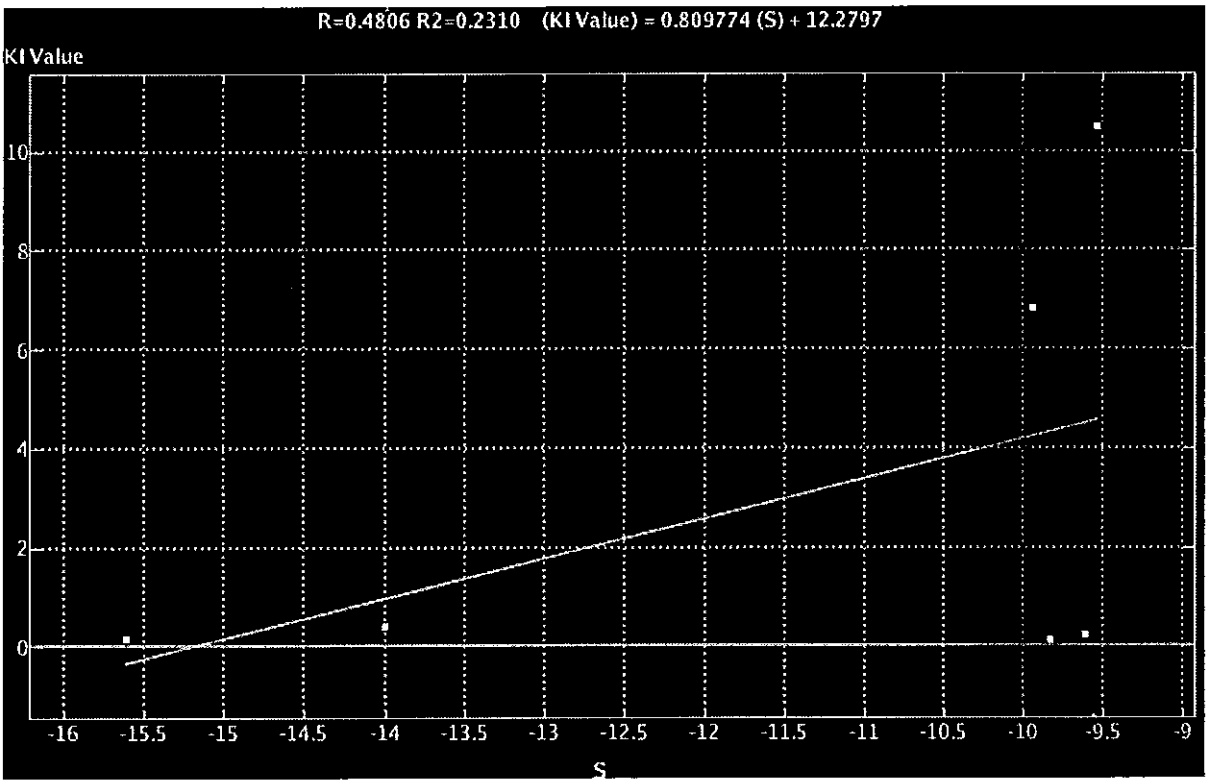


Figure 11: Correlation plot: K_i vs. S for ligands in ER Alpha (Tamoxifen excluded)

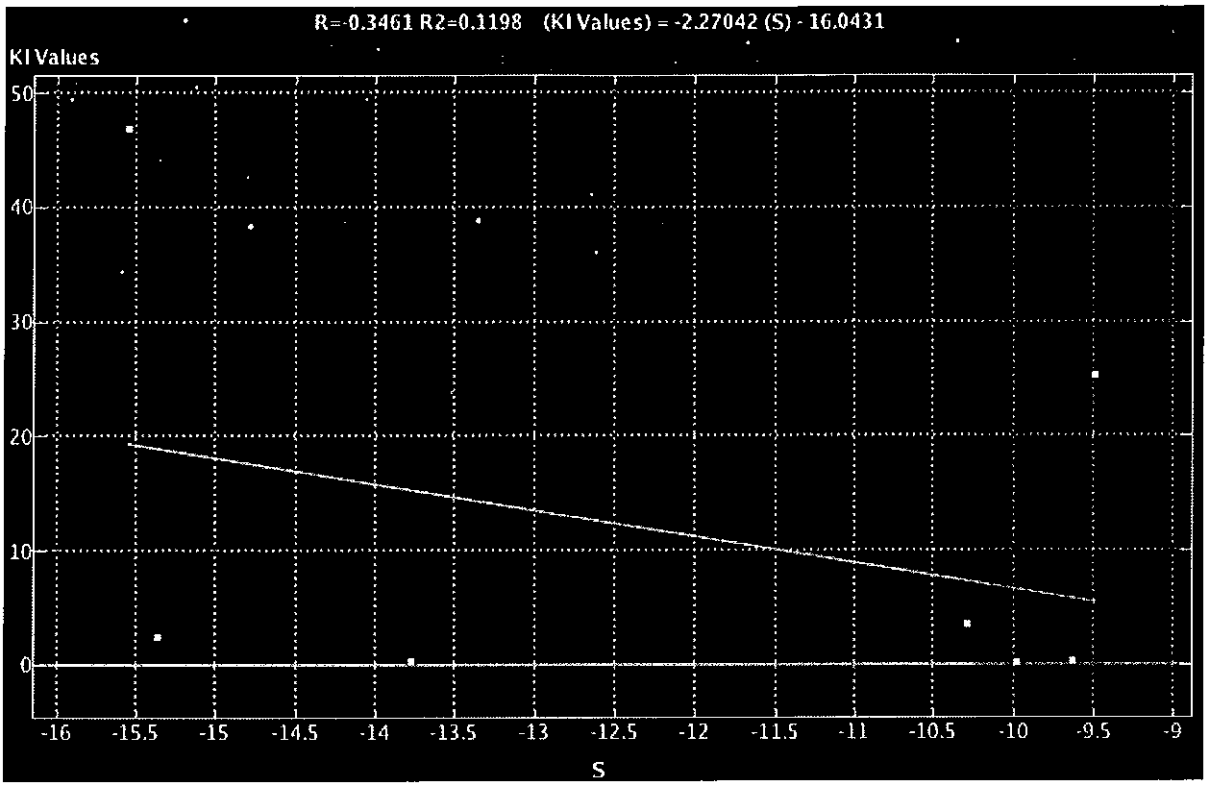


Figure 12: Correlation plot: K_i vs. S for ligands in ER Beta (Tamoxifen included)

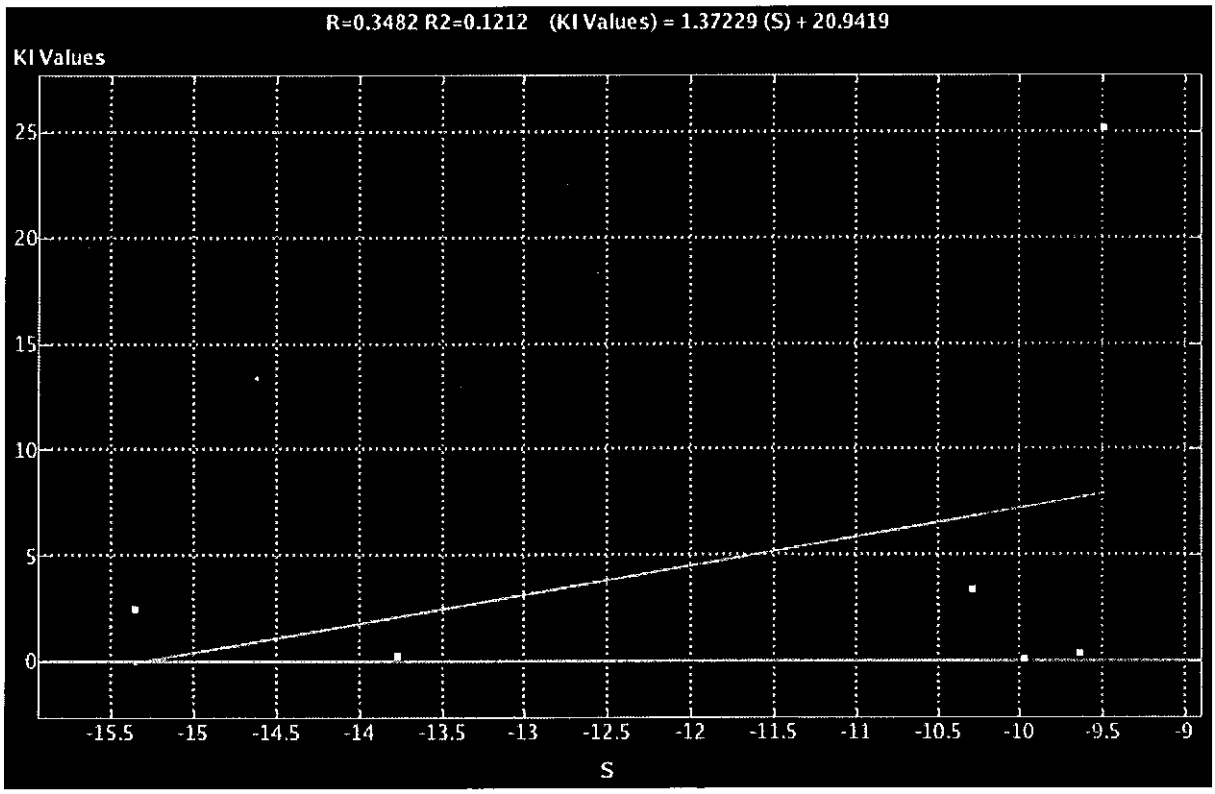
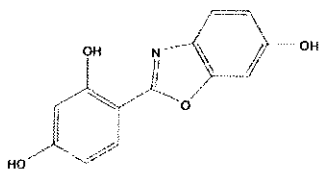
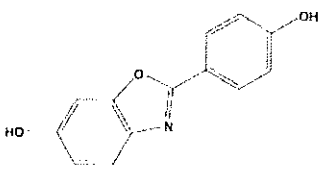


Figure 13: Correlation plot: K_i vs. S for ligands in ER Beta (Tamoxifen excluded)

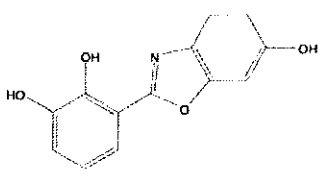
1



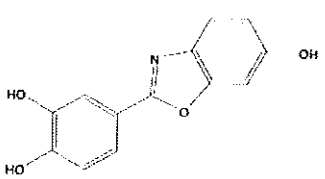
2



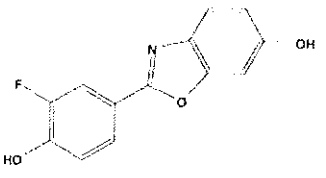
3



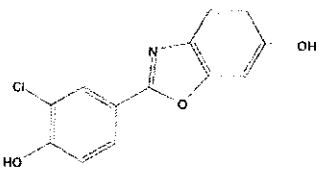
4



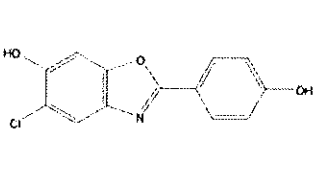
5



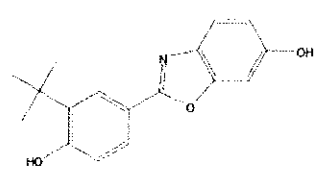
6



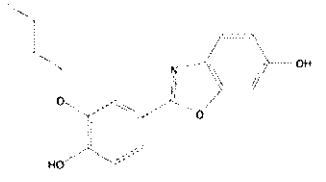
7



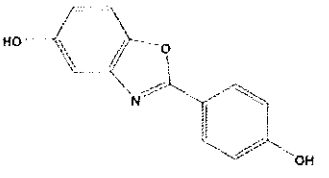
8



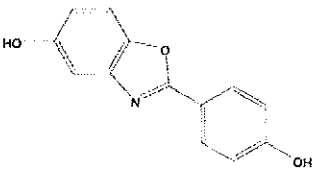
9



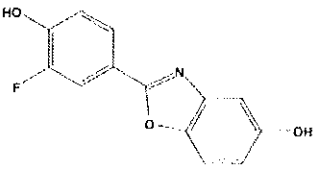
10



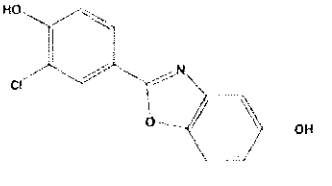
11



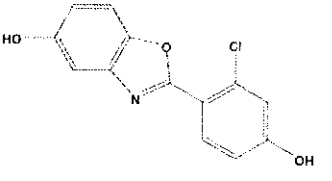
12



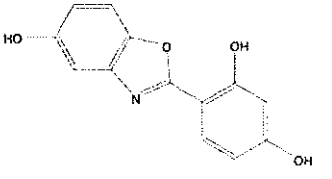
13



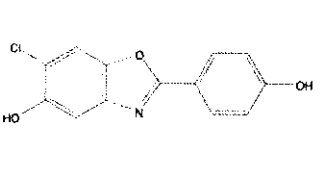
14



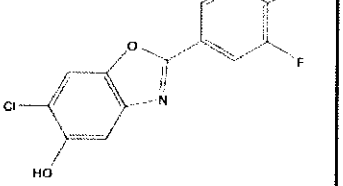
15



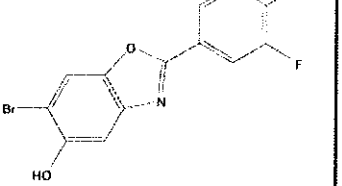
16



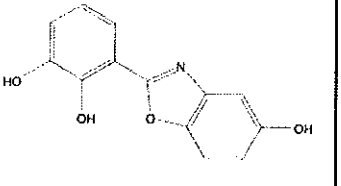
17



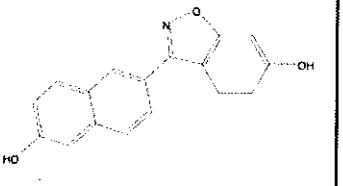
18



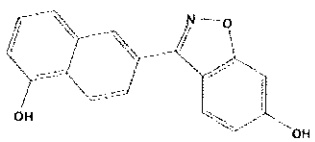
19



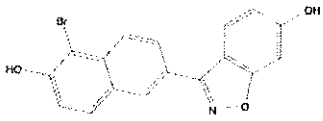
20



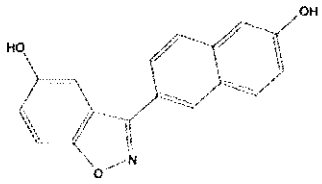
21



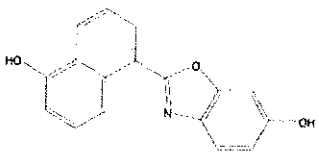
22



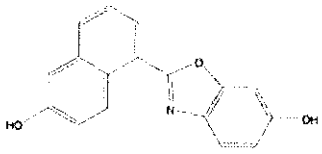
23



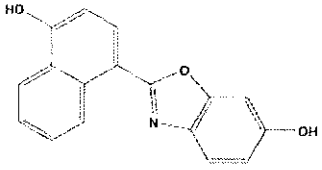
24



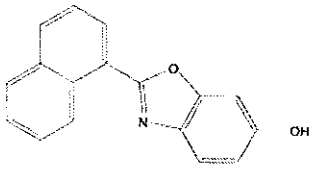
25



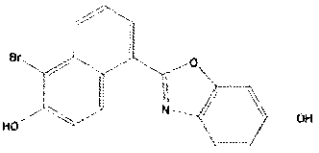
26



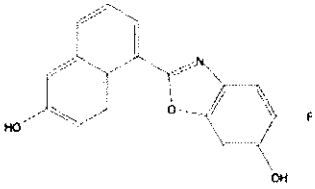
27



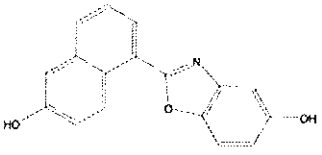
28



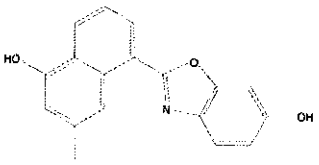
29



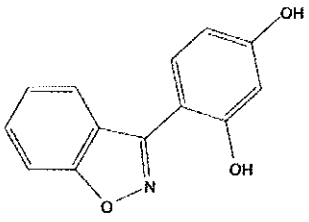
30



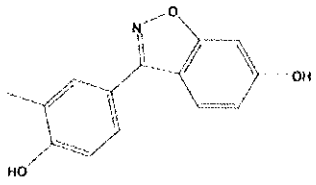
31



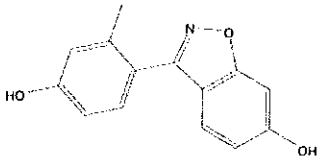
32



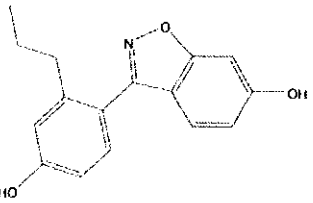
33



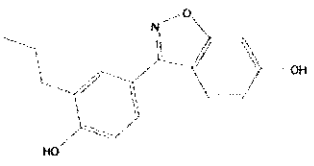
34



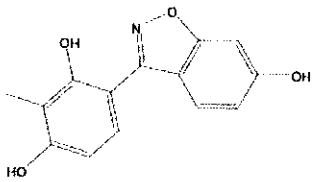
35



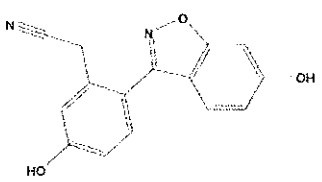
36



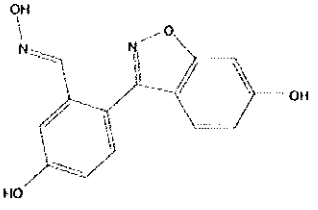
37



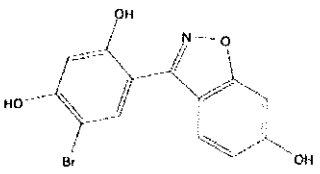
38



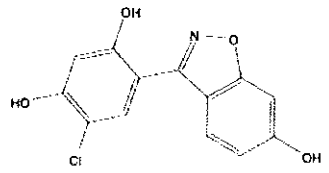
39



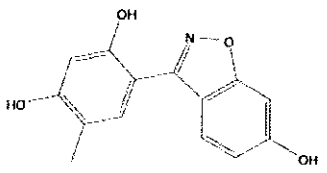
40



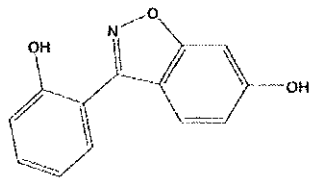
41



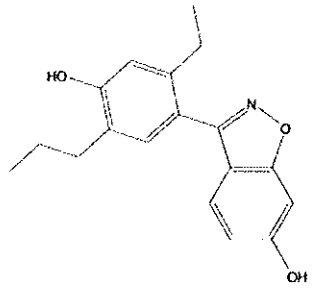
42



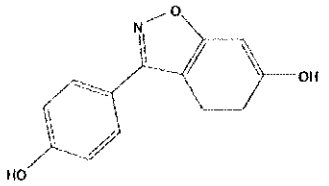
43



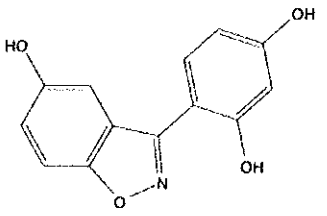
44



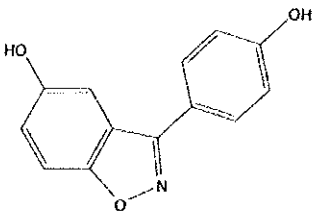
45



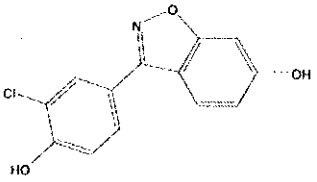
46



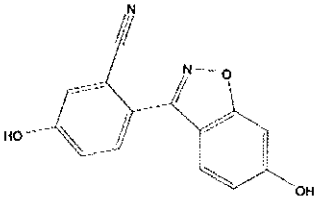
47



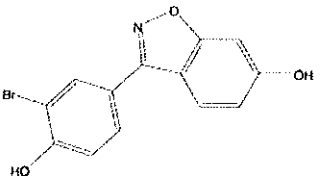
48



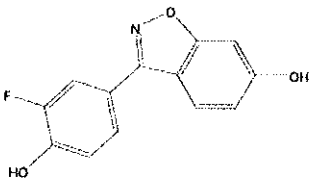
49



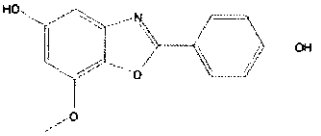
50



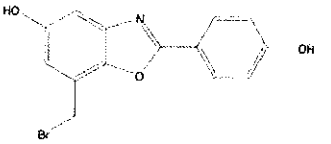
51



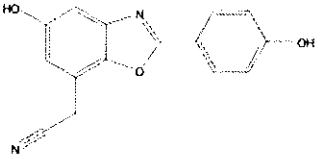
52



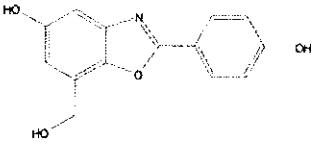
53



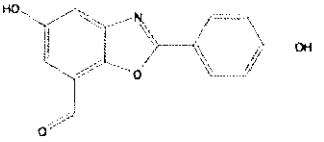
54



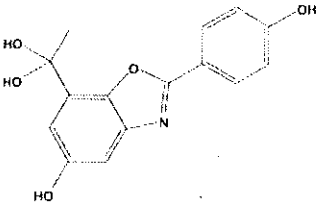
55



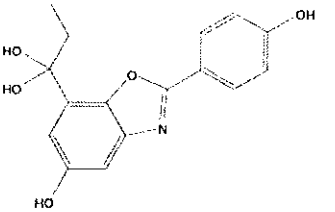
56



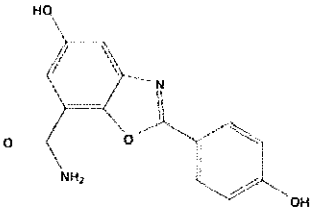
57



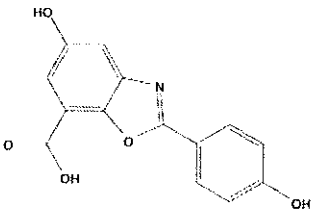
58



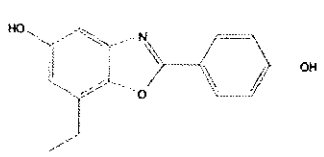
59



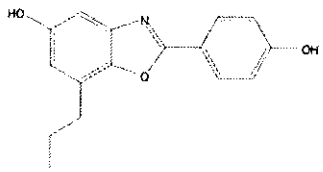
60



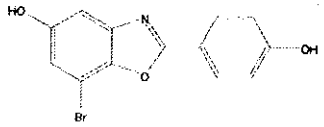
61



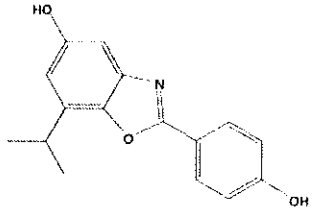
62



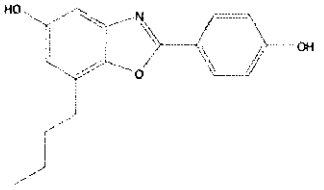
63



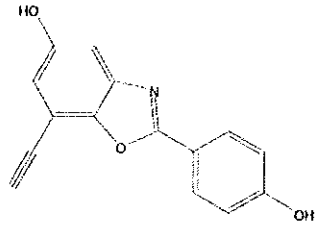
64



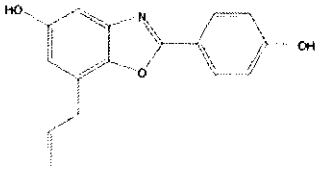
65



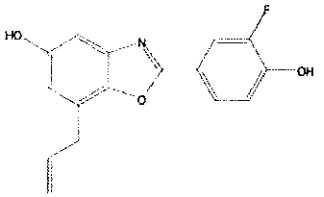
66



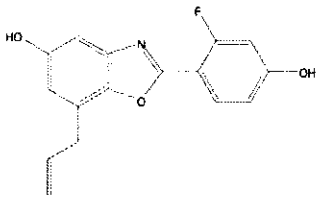
67



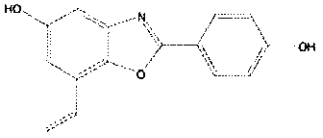
68



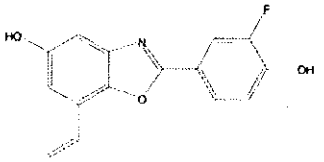
69



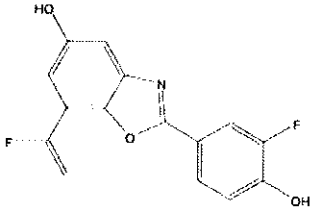
70



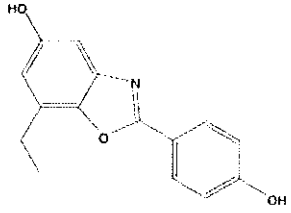
71



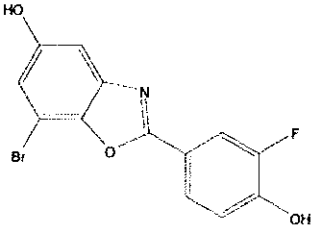
72



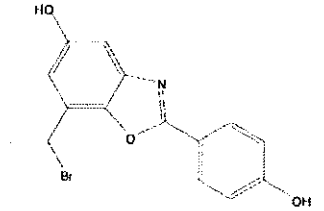
73



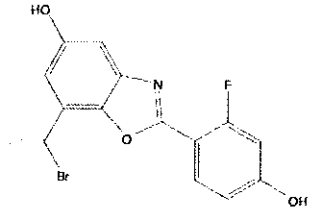
74



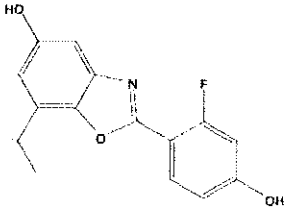
75



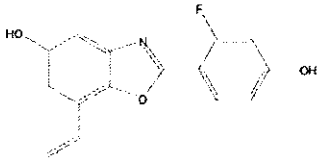
76



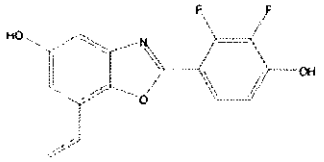
77



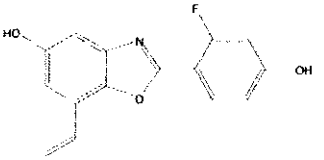
78



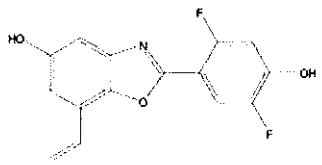
79



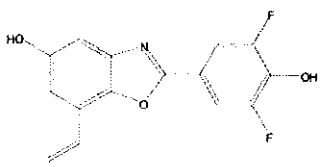
80



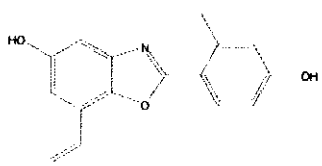
81



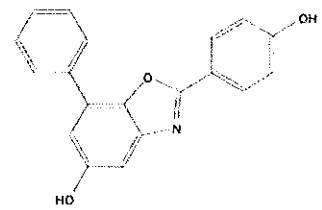
82



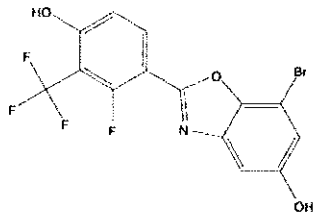
83



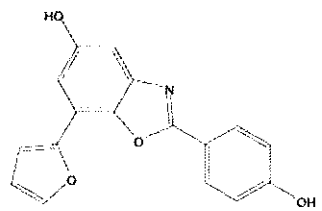
84



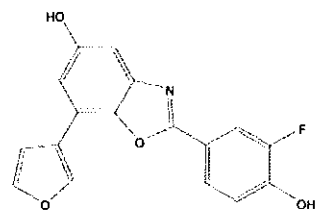
85



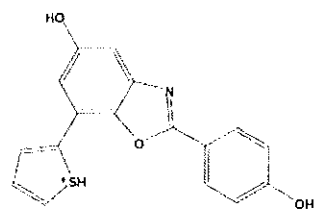
86



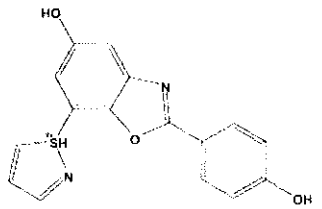
87



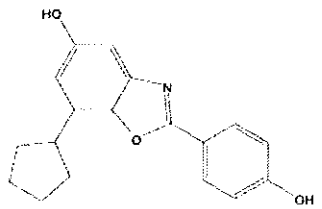
88



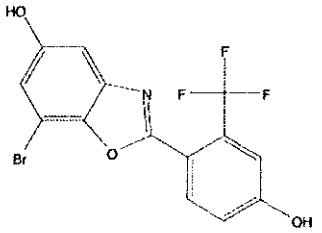
89



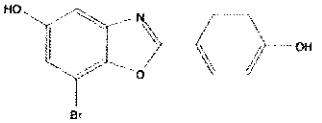
90



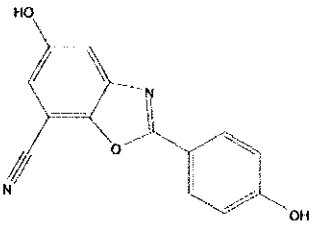
91



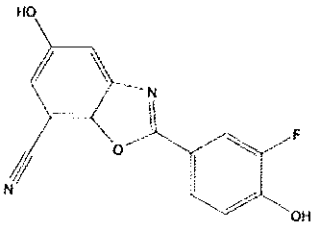
92



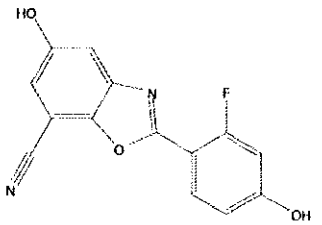
93



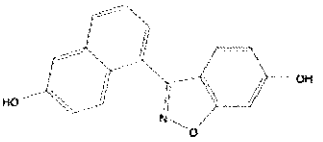
94



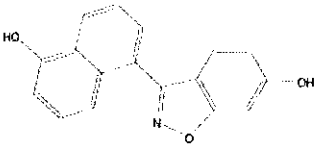
95



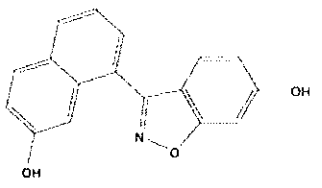
96



97

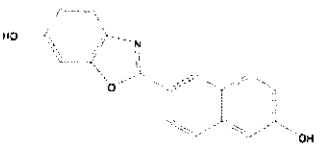


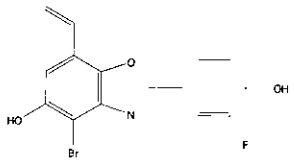
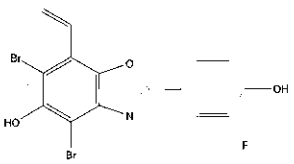
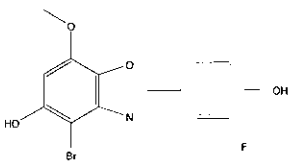
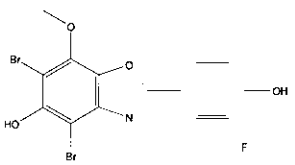
98

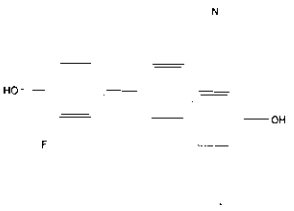
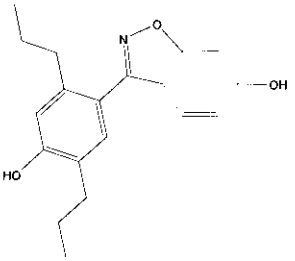


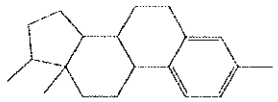
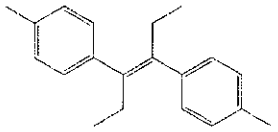
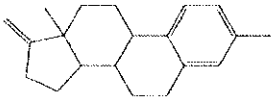
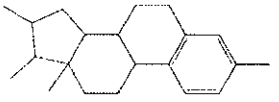
99

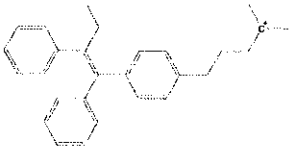
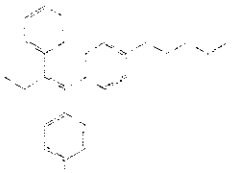
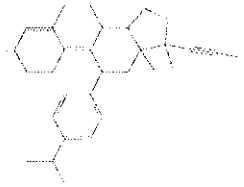
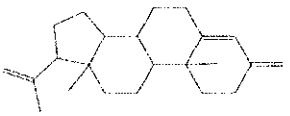
100



101	102	103	104
			

105	106
	

	mol	Molecule
1		17 Beta-Estradiol
2		Diethylstilbestrol
3		Estrone
4		Estriol
5		Raloxifene

	mol	Molecule
6		Tamoxifen
7		4-OH-Tamoxifen
8		RU486
9		Progesterone

References

1. Matsushima, A., et al., *Bisphenol AF Is a Full Agonist for the Estrogen Receptor ER α but a Highly Specific Antagonist for ERβ.* Environ Health Perspect, 2010. **118**(9).
2. Escande, A., et al., *Evaluation of ligand selectivity using reporter cell lines stably expressing estrogen receptor alpha or beta.* Biochemical Pharmacology, 2006. **71**(10): p. 1459-1469.
3. Kuiper Gg Fau - Shughrue, P.J., et al., *The estrogen receptor beta subtype: a novel mediator of estrogen action in neuroendocrine systems.* (0091-3022 (Print)).
4. Pike, A.C.W., et al., *Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist.* EMBO J, 1999. **18**(17): p. 4608-4618.
5. Métivier, R., et al., *Function of N-Terminal Transactivation Domain of the Estrogen Receptor Requires a Potential α-Helical Structure and Is Negatively Regulated by the A Domain.* Molecular Endocrinology, 2000. **14**(11): p. 1849-1871.
6. Pettersson, K. and J.-A. Gustafsson, *ROLE OF ESTROGEN RECEPTOR BETA IN ESTROGEN ACTION.* Annual Review of Physiology, 2001. **63**(1): p. 165.
7. Wurtz, J.-M., et al., *A canonical structure for the ligand-binding domain of nuclear receptors.* Nat Struct M(Biol, 1996. **3**(1): p. 87-94.
8. Younes M Fau - Honma, N. and N. Honma, *Estrogen receptor beta.* (1543-2165 (Electronic)).
9. Gustafsson, J.A., *Estrogen receptor beta--a new dimension in estrogen mechanism of action.* Journal of Endocrinology, 1999. **163**(3): p. 379-383.
10. Tonetti, D.A. and V.C. Jordan, *The Estrogen Receptor: A Logical Target for the Prevention of Breast Cancer with Antiestrogens.* Journal of Mammary Gland Biology and Neoplasia, 1999. **4**(4): p. 401-413.
11. Belcher, S.M. and A. Zsarnovszky, *Estrogenic Actions in the Brain: Estrogen, Phytoestrogens, and Rapid Intracellular Signaling Mechanisms.* Journal of Pharmacology and Experimental Therapeutics, 2001. **299**(2): p. 408-414.
12. Luan, F., et al., *Classification of estrogen receptor-β ligands on the basis of their binding affinities using support vector machine and linear discriminant analysis.* European Journal of Medicinal Chemistry, 2008. **43**(1) p. 43-52.
13. Mewshaw Re Fau - Edsall, R.J., Jr., et al., *ERbeta ligands. 3. Exploiting two binding orientations of the 2-phenylnaphthalene scaffold to achieve ERbeta selectivity.* (0022-2623 (Print)).
14. Zou A Fau - Marschke, K.B., et al., *Estrogen receptor beta activates the human retinoic acid receptor alpha promoter in response to tamoxifen and other estrogen receptor antagonists, but not in response to estrogen.* (088 8809 (Print)).
15. Brzozowski Am Fau - Pike, A.C., et al., *Molecular basis of agonism and antagonism in the oestrogen receptor.* (0028-0836 (Print)).
16. Wiseman H Fau - Lewis, D.F. and D.F. Lewis, *The metabolism of tamoxifen by human cytochromes P450 is rationalized by molecular modelling of the enzyme-substrate interactions: potential importance to its proposed anti-carcinogenic/carcinogenic actions.* (0143-3334 (Print)).
17. Wang, D.-Y., et al., *Identification of Estrogen-Responsive Genes by Complementary Deoxyribonucleic Acid Microarray and Characterization of a Novel Early Estrogen-Induced Gene: EEIG1.* Molecular Endocrinology, 200- **18**(2): p. 402-411.